

	L #	Hits	Search Text
1	L1	1346	glycosidase\$2
2	L2	3660	beta adj 2 1
3	L3	35	thermophillic
4	L4	6145	100 adj2 c
5	L5	35	2 and 4
6	L6	157	pyrococcus
7	L7	0	5 and 6
8	L8	1	ph adj3 6
9	L9	79	beta adj2 glycosidase\$2
10	L10	4	9 and 4
11	L11	4744	4 and temperature
12	L13	0	12 and 6
13	L12	32	2 and 11
14	L14	10	12 and optimum

had a half-life of > 60 h at 90 degree C. Although the enzyme is much less thermostable than the β -glucosidase, which had a reported half-life of 85 h at 100 degree C. $K_{sub}(m)$ and $V_{sub}(max)$ values for the β -mannosidase were determined to be 0.79 mM and 31.1 μ mol para-nitrophenol released/min/mg with p-nitrophenyl- β -D-mannopyranoside as substrate. The catalytic efficiency of the β -mannosidase was significantly lower than that reported for the P. furiosus β -glucosidase (5.3 versus 4, 500 $s^{-1}mM^{-1}$ with p-nitrophenyl- β -D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the β -glucosidase, the primary role of the β -mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

=> d his

(FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000

```

L1      95500 S GALACTOSIDASE?
L2      80870 S BETA(W)L1
L3      349 S B(W)L1
L4      81054 S L2 OR L3
L5      87 S THERMOPHILIC
L6      1 S L5 AND (L1 OR L4)
L7      86 S L1 AND (100 (2W)C)
L8      3787 S PYROCOCOCCUS
L9      7 S L7 AND L8
L10     3 DUP REM L9 (4 DUPLICATES REMOVED)
L11     2 S L10 AND PH
L12     23070 S GLYCOSIDASE?
L13     1110 S BETA(W)L12
L14     65 S B(2W)L12
L15     1169 S L13 OR L14
L16     0 S L15 AND L5
L17     0 S L12 AND L5
L18     77 S L8 AND L12
L19     26 S L18 AND (100(2W)C)
L20     15 S L19 AND PH

```

=> dup rem

ENTER L# LIST OR (END):l20

PROCESSING COMPLETED FOR L20

L21 6 DUP REM L20 (9 DUPLICATES REMOVED)

=> d 1-6 ibib ab

```

L21  ANSWER 1 OF 6  MEDLINE                                     DUPLICATE 1
ACCESSION NUMBER:  2000141228      MEDLINE
DOCUMENT NUMBER:   20141228
TITLE:             Novel substrate specificity of a membrane-bound beta-
                    glycosidase from the hyperthermophilic archaeon
                    Pyrococcus horikoshii.
AUTHOR:            Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y;
                    Honda K
CORPORATE SOURCE:  National Institute of Bioscience and Human-Technology,
                    Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
SOURCE:            FEBS LETTERS, (2000 Feb 11) 467 (2-3) 195-200.

```

L20 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 2000:95843 HCAPLUS

TITLE: Novel substrate specificity of a membrane-bound
.beta.-**glycosidase** from the
hyperthermophilic archaeon **Pyrococcus**
horikoshii

AUTHOR(S): Matsui, I.; Sakai, Y.; Matsui, E.; Kikuchi, H.;
Kawarabayasi, Y.; Honda, K.

CORPORATE SOURCE: National Institute of Bioscience and
Human-Technology,

TSUKUBA, IBARAKI, JAPAN
SOURCE: FEBS Lett. (2000), 467(2,3), 195-200

CODEN: FEBLAL; ISSN: 0014-5793

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A .beta.-**glycosidase** gene homolog of **Pyrococcus**
horikoshii (BGPh) was successfully expressed in Escherichia coli. The
enzyme was localized in a membrane fraction and solubilized with 2.5%
Triton X-100 at 85.degree.C for 15 min. The optimum pH was 6.0
and the optimum temp. was over 100.degree.C, resp.
BGPh stability was dependent on the presence of Triton X-100, the
enzyme's
half-life at 90.degree.C (pH 6.0) was 15 h. BGPh has a novel
substrate specificity with kcat/Km values high enough for hydrolysis of
.beta.-D-Glcp derivs. with long alkyl chain at the reducing end and low
enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic
than aryl- or alkyl-.beta.-D-Glcp.

late -

L20 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1997:170983 BIOSIS

DOCUMENT NUMBER: PREV199799477586

TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaeobacterium

Pyrococcus abyssi: Purification and characterization.

AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George

CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France

SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.

ISSN: 0941-2905.

DOCUMENT TYPE: Article

LANGUAGE: English

AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaeobacterium **Pyrococcus** abyssi strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture

medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal

activity at pH 6 and 100 degree C and was highly thermostable. It exhibited different beta-glycosidase activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group

was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked

contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

Welcome to STN International! Enter x:x

LOGINID:ssspta1652mxm

PASSWORD:

* * * * * RECONNECTED TO STN INTERNATIONAL * * * * *

SESSION RESUMED IN FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS,

NTIS, LIFESCI' AT 09:16:54 ON 10 MAY 2000

FILE 'MEDLINE' ENTERED AT 09:16:54 ON 10 MAY 2000

FILE 'EMBASE' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 Elsevier Science B.V. All rights reserved.

FILE 'BIOSIS' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 BIOSIS(R)

FILE 'BIOTECHDS' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 DERWENT INFORMATION LTD

FILE 'SCISEARCH' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 Institute for Scientific Information (ISI) (R)

FILE 'HCAPLUS' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'NTIS' ENTERED AT 09:16:54 ON 10 MAY 2000

All rights reserved. (2000)

FILE 'LIFESCI' ENTERED AT 09:16:54 ON 10 MAY 2000

COPYRIGHT (C) 2000 Cambridge Scientific Abstracts (CSA)

d

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

23.66

23.81

=> his

HIS IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.

For a list of commands available to you in the current file, enter

"HELP COMMANDS" at an arrow prompt (=>).

=> s glycosidase?

7 FILES SEARCHED...

L12 23070 GLYCOSIDASE?

=> d his

(FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000

L1 95500 S GALACTOSIDASE?
L2 80870 S BETA(W)L1
L3 349 S B(W)L1
L4 81054 S L2 OR L3
L5 87 S THERMOPHILLIC
L6 1 S L5 AND (L1 OR L4)
L7 86 S L1 AND (100 (2W)C)
L8 3787 S PYROCOCCLUS
L9 7 S L7 AND L8
L10 3 DUP REM L9 (4 DUPLICATES REMOVED)
L11 2 S L10 AND PH

L12 23070 S GLYCOSIDASE?

=> s beta(w)l12

L13 1110 BETA(W) L12

=> s b(2w)l12

L14 65 B(2W) L12

=> s l13 or l14

L15 1169 L13 OR L14

=> s l15 and l5

L16 0 L15 AND L5

=> s l12 and l5

L17 0 L12 AND L5

=> s l8 and l12

L18 77 L8 AND L12

=> s l18 and (100(2w)C)

L19 26 L18 AND (100(2W) C)

=> s l19 and ph

6 FILES SEARCHED...

L20 15 L19 AND PH

=> dup rem

ENTER L# LIST OR (END):120

COMMAND INTERRUPTED

If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> dup rem

ENTER L# LIST OR (END):119

COMMAND INTERRUPTED

If this message appears repeatedly, please notify the Help Desk.
Enter "HELP STN" for information on contacting the nearest STN Help
Desk by telephone or via SEND in the STNMAIL file.

=> d his

(FILE 'HOME' ENTERED AT 08:37:47 ON 10 MAY 2000)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 08:38:10 ON 10 MAY 2000

L1 95500 S GALACTOSIDASE?
L2 80870 S BETA(W)L1
L3 349 S B(W)L1
L4 81054 S L2 OR L3
L5 87 S THERMOPHILIC

L6 1 S L5 AND L1 OR L4)
 L7 86 S L1 AND L2 (2W)C)
 L8 3787 S PYROCOCCLUS
 L9 7 S L7 AND L8
 L10 3 DUP REM L9 (4 DUPLICATES REMOVED)
 L11 2 S L10 AND PH
 L12 23070 S GLYCOSIDASE?
 L13 1110 S BETA(W)L12
 L14 65 S B(2W)L12
 L15 1169 S L13 OR L14
 L16 0 S L15 AND L5
 L17 0 S L12 AND L5
 L18 77 S L8 AND L12
 L19 26 S L18 AND (100(2W)C)
 L20 15 S L19 AND PH

=> d 1-15 ibib ab

L20 ANSWER 1 OF 15 MEDLINE
 ACCESSION NUMBER: 2000141228 MEDLINE
 DOCUMENT NUMBER: 20141228
 TITLE: Novel substrate specificity of a membrane-bound beta-
glycosidase from the hyperthermophilic archaeon
Pyrococcus horikoshii.
 AUTHOR: Matsui I; Sakai Y; Matsui E; Kikuchi H; Kawarabayasi Y;
 Honda K
 CORPORATE SOURCE: National Institute of Bioscience and Human-Technology,
 Tsukuba, Ibaraki, Japan.. ikmatsui@nibh.go.jp
 SOURCE: FEBS LETTERS, (2000 Feb 11) 467 (2-3) 195-200.
 Journal code: EUH. ISSN: 0014-5793.
 PUB. COUNTRY: Netherlands
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; Cancer Journals
 ENTRY MONTH: 200006
 ENTRY WEEK: 20000602
 AB A beta-**glycosidase** gene homolog of **Pyrococcus**
 horikoshii (BGPh) was successfully expressed in Escherichia coli. The
 enzyme was localized in a membrane fraction and solubilized with 2.5%
 Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0
 and the optimum temperature was over 100 degrees C,
 respectively. BGPh stability was dependent on the presence of Triton
 X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h.
 BGPh has a novel substrate specificity with k(cat)/K(m) values high
 enough
 for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the
 reducing end and low enough for the hydrolysis of beta-linked glucose
 dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L20 ANSWER 2 OF 15 MEDLINE
 ACCESSION NUMBER: 96394494 MEDLINE
 DOCUMENT NUMBER: 96394494
 TITLE: Comparison of a beta-glucosidase and a beta-mannosidase
 from the hyperthermophilic archaeon **Pyrococcus**
 furiosus. Purification, characterization, gene cloning,
 and
 sequence analysis.
 AUTHOR: Bauer M W; Bylina E J; Swanson R V; Kelly R M
 CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State
 University, Raleigh, North Carolina 27695-7905, USA.
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 27) 271 (39)
 23749-55.
 Journal code: HIV. ISSN: 0021-9258.
 PUB. COUNTRY: United States

Journal Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-U60214
ENTRY MONTH: 199701

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon *Pyrococcus furiosus*: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-glycosidases from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from *P. furiosus* both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at pH 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at 100 degrees C. Km and Vmax values for the beta-mannosidase were determined to be 0.79 mM

and

31.1 micromol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the *P. furiosus* beta-glucosidase (5.3 versus 4, 500 s⁻¹ mM⁻¹ with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 3 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2000055777 EMBASE

TITLE: Novel substrate specificity of a membrane-bound .beta.-glycosidase from the hyperthermophilic archaeon *Pyrococcus horikoshii*.

AUTHOR: Matsui I.; Sakai Y.; Matsui E.; Kikuchi H.; Kwarabayasi Y.; Honda K.

CORPORATE SOURCE: I. Matsui, National Institute, Bioscience and Human Technology, Tsukuba, Ibaraki 305, Japan.
ikmatsui@nibh.go.jp

SOURCE: FEBS Letters, (2000) 467/2-3 (195-200).

Refs: 32

ISSN: 0014-5793 CODEN: FEBLAL

PUBLISHER IDENT.: S 0014-5793(00)01156-X

COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB A .beta.-glycosidase gene homolog of *Pyrococcus horikoshii* (BGPh) was successfully expressed in *Escherichia coli*. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85.degree.C for 15 min. The optimum pH was 6.0

and the optimum temperature was over 100.degree.C, respectively. BGPh's stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90.degree.C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with $k(\text{cat})/K(\text{m})$ values high enough for hydrolysis of .beta.-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic than aryl- or alkyl-.beta.-D-Glcp. Copyright (C) 2000 Federation of European Biochemical Societies.

L20 ANSWER 4 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable .beta.-**glycosidase** from **Pyrococcus kodakaraensis** KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A .beta.-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus kodakaraensis** KOD1. The gene encoding .beta.-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other .beta.-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to .beta.-mannosidase from *P. furiosus* (55.7%), .beta.-**glycosidase** from *Sulfolobus solfataricus* (42.7%) and .beta.-glucosidase from *P. furiosus* (41.9%). The cloned gene was expressed in *Escherichia coli* and the recombinant protein was purified. The .beta.-**glycosidase** showed optimal activity at pH 6.5 and at an extremely high temperature of 100.degree.C, and had a half-life of 18 h at 90.degree.C. The .beta.-**glycosidase** hydrolyzed various pNp-.beta.-glycopyranosides, with $k(\text{cat})/K(\text{m})$ values in the order of pNp-.beta.-glucopyranoside > pNp-.beta.-mannopyranoside > pNp-.beta.-galactopyranoside > pNp-.beta.-xylopyranoside. pNp-.beta.-mannopyranoside was the substrate exhibiting the lowest $K(\text{m})$ value [0.254 mM] with a $k(\text{cat})/K(\text{m})$ ratio comparable to that of pNp-.beta.-glucopyranoside. This substrate specificity was distinct from previously reported .beta.-**glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth .alpha.-helix and .beta.-strand region of .beta.-**glycosidase** from *S. solfataricus*, which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to .beta.-**glycosidases** and the C-terminal region similar to .beta.-mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L20 ANSWER 5 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97196266 EMBASE

DOCUMENT NUMBER: 1997100266
TITLE: Cloning, sequencing, characterization, and expression of
an extracellular .alpha.-amylase from the hyperthermophilic
archaeon **Pyrococcus** furiosus in Escherichia coli
and Bacillus subtilis.
AUTHOR: Jorgensen S.; Vorgias C.E.; Antranikian G.
CORPORATE SOURCE: G. Antranikian, Inst. of Biotechnology, Dept. of Technical
Microbiology, Technical University Hamburg-Harburg,
Denickestr. 15, 21071 Hamburg, Germany
SOURCE: Journal of Biological Chemistry, (1997) 272/26
(16335-16342).
Refs: 27
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A gene encoding a highly thermostable extracellular .alpha.-amylase from
the hyperthermophilic archaeon **Pyrococcus** furiosus was
identified. The gene was cloned, sequenced, and expressed in Escherichia
coli and Bacillus subtilis. The gene is 1383 base pairs long and encodes

a protein of 461 amino acids. The open reading frame of the gene was
verified by microsequencing of the recombinant purified enzyme. The
deduced amino acid sequence is 25 amino acids longer at the N terminus
than that determined by sequencing of the purified protein, suggesting
that a leader sequence is removed during transport of the enzyme across
the membrane. The recombinant .alpha.-amylase was biochemically
characterized and shows an activity optimum at pH 4.5, whereas
the optimum temperature for enzymatic activity is close to 100
.degree.C. .alpha.-Amylase shows sequence homology to the other
known .alpha.-amylases and belongs to family 13 of glycosyl hydrolases.
This extracellular .alpha.-amylase is not homologous to the subcellular
.alpha.-amylase previously isolated from the same organism.

L20 ANSWER 6 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 96300283 EMBASE

DOCUMENT NUMBER: 1996300283

TITLE: Comparison of a .beta.-glucosidase and a
.beta.-mannosidase

from the hyperthermophilic archaeon **Pyrococcus**
furiosus. Purification, characterization, gene cloning,
and sequence analysis.

AUTHOR: Bauer M.W.; Bylina E.J.; Swanson R.V.; Kelly R.M.
CORPORATE SOURCE: Dept. of Chemical Engineering, North Carolina State
University, Raleigh, NC 27695-7905, United States

SOURCE: Journal of Biological Chemistry, (1996) 271/39
(23749-23755).

ISSN: 0021-9258 CODEN: JBCHA3

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Two distinct exo-acting, .beta.-specific glycosyl hydrolases were
purified
to homogeneity from crude cell extracts of the hyperthermophilic archaeon
Pyrococcus furiosus: a .beta.-glucosidase, corresponding to the
one previously purified by Kengen et al (Kengen, S. W. M., Luesink, E.

J.,
Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213,

305-312), and a .beta.-mannosidase. The .beta.-mannosidase and .beta.-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amine acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the .beta.-mannosidase and the .beta.-glucosidase, respectively. The .beta.-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J.,

and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557). The deduced amine acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the .beta.-glycosidases from Sulfolobus solfataricus, Thermotoga maritima, and Caldocellum saccharolyticum. Based on these sequence similarities, the .beta.-mannosidase and the .beta.-glucosidase can both be classified as family I glycosyl hydrolases. In addition, the .beta.-mannosidase and .beta.-glucosidase from P. furiosus both contained the conserved active site residues found in all family I enzymes. The .beta.-mannosidase showed optimal activity at pH 7.4 and 105 .degree.C. Although the enzyme had a half-life of greater than 60 h at 90 .degree.C, it is much less thermostable than the .beta.-glucosidase, which had a reported half-life of 85 h at 100 .degree.C. Km and V(max) values for the .beta.-mannosidase were determined to be 0.79 mM and 31.1 .mu.mol para-nitrophenol released/min/mg with p-nitrophenyl-.beta.-D-mannopyranoside as substrate. The catalytic efficiency of the .beta.-mannosidase was significantly lower than that reported for the P. furiosus .beta.-glucosidase (5.3 versus 4, 500 s-1 mM-1 with p-nitrophenyl-B-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the .beta.-glucosidase, the primary role of the .beta.-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 7 OF 15 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 90219204 EMBASE

DOCUMENT NUMBER: 1990219204

TITLE: Purification and characterization of an .alpha.-glucosidase

from a hyperthermophilic archaebacterium, **Pyrococcus** furiosus, exhibiting a temperature optimum of 105 to 115.degree.C.

AUTHOR: Costantino H.R.; Brown S.H.; Kelly R.M.

CORPORATE SOURCE: Department of Chemical, Engineering, Johns Hopkins University, Baltimore, MD 21218, United States

SOURCE: Journal of Bacteriology, (1990) 172/7 (3654-3660). ISSN: 0021-9193 CODEN: JOBAAJ

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Pyrococcus** furiosus is a strictly anaerobic hyperthermophilic archaebacterium with an optimal growth temperature of about 100 .degree.C. When this organism was grown in the presence of certain complex carbohydrates, the production of several amylolytic enzymes was noted. These enzymes included an .alpha.-glycosidase that was located in the cell cytoplasm. This .alpha.-glucosidase has been purified 310-fold and corresponded to a protein band of 125 kilodaltons

as

resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The enzyme exhibited optimum activity at pH 5.0 to 6.0 and over a temperature range of 105 to 115.degree.C. Kinetic analysis conducted at 108.degree.C revealed hydrolysis of the substrate p-nitrophenyl-.alpha.-D-

glucopyranoside (PNPG), methyl-.alpha.-D-glucopyranoside, maltose, and isomaltose. Trace activity was detected towards p-nitrophenyl-.beta.-D-glucopyranoside, and no activity could be detected towards starch or sucrose. Inhibition studies conducted at 108.degree.C with PNPG as the substrate and maltose as the inhibitor yielded a K(i) for maltose of 14.3 mM. Preincubation for 30 min at 98.degree.C in 100 mM dithiothreitol and 1.0 M urea had little effect on enzyme activity, whereas preincubation in 1.0% sodium dodecyl sulfate and 1.0 M guanidine hydrochloride resulted in significant loss of enzyme activity. Purified .alpha.-glycosidase from *P. furiosus* exhibited remarkable thermostability; incubation of the enzyme at 98.degree.C resulted in a half life of nearly 48 h.

L20 ANSWER 8 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1997:170983 BIOSIS

DOCUMENT NUMBER: PREV199799477586

TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaeobacterium *Pyrococcus abyssi*: Purification and characterization.

AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George

CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France

SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.
ISSN: 0941-2905.

DOCUMENT TYPE: Article

LANGUAGE: English

AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaeobacterium *Pyrococcus abyssi* strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal activity at pH 6 and 100 degree C and was highly thermostable. It exhibited different beta-glycosidase activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

L20 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1996:508084 BIOSIS

DOCUMENT NUMBER: PREV199699230440

TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon *Pyrococcus furiosus*: Purification, characterization, gene cloning and sequence analysis.

AUTHOR(S): Bauer, Michael W.; Bylina, Edward J.; Swanson, Ronald V.; Kelly, Robert M. (1)

CORPORATE SOURCE: (1) Dep. Chem. Engineering, North Carolina State Univ., Raleigh, NC 27695-7905 USA

SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 39, pp. 23749-23755.
ISSN: 0021-9258.

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus furiosus**: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557).

The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-glycosidases from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from *P. furiosus* both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at pH 7.4 and 105 degree C. Although the enzyme had a half-life of greater than 60 h at 90 degree C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at 100 degree C. K-m and V-max values for the beta-mannosidase were determined to be 0.79 mM and 31.1 mu-mol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the *P. furiosus* beta-glucosidase (5.3 versus 4,500 s-1 mM-1 with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 10 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)

ACCESSION NUMBER: 2000:156669 SCISEARCH

THE GENUINE ARTICLE: 285ZF

TITLE: Novel substrate specificity of a membrane-bound beta-glycosidase from the hyperthermophilic archaeon **Pyrococcus horikoshii**

AUTHOR: Matsui I (Reprint); Sakai Y; Matsui E; Kikuchi H; Kawarabayashi Y; Honda K

CORPORATE SOURCE: NATL INST BIOSCI & HUMAN TECHNOL, TSUKUBA, IBARAKI 305, JAPAN (Reprint); MINIST INT TRADE & IND, NATL INST

TECHNOL & EVALUAT, TOKYO, JAPAN

COUNTRY OF AUTHOR: JAPAN

SOURCE: FEBS LETTERS, (11 FEB 2000) Vol. 467, No. 2-3, pp. 195-200

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
 ISSN: 0014-5793.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A beta-**glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in *Escherichia coli*. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min, The optimum pH was 6.0 and the optimum temperature was over 100 degrees C, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h, BGPh has a novel substrate specificity with k(cat)/K-m values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp (C) 2000 Federation of European Biochemical Societies.

L20 ANSWER 11 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)

ACCESSION NUMBER: 1999:834444 SCISEARCH

THE GENUINE ARTICLE: 249NG

TITLE: Gene analysis and enzymatic properties of thermostable-beta-**glycosidase** from **Pyrococcus** kodakaraensis KOD1

AUTHOR: Ezaki S; Miyaoku K; Nishi K I; Tanaka T; Fujiwara S; Takagi M; Atomi H; Imanaka T (Reprint)

CORPORATE SOURCE: KYOTO UNIV, GRAD SCH ENGN, DEPT SYNTHET CHEM & BIOL CHEM, SAKYO KU, YOSHIDA HONMACHI, KYOTO 6068501, JAPAN (Reprint); KYOTO UNIV, GRAD SCH ENGN, DEPT SYNTHET CHEM & BIOL CHEM, SAKYO KU, KYOTO 6068501, JAPAN; OSAKA UNIV, GRAD SCH ENGN, DEPT BIOTECHNOL, SUITA, OSAKA 5650871, JAPAN; KANEKA CORP, FINE CHEM RES LABS, TAKASAGO, HYOGO 6768688, JAPAN

COUNTRY OF AUTHOR: JAPAN

SOURCE: JOURNAL OF BIOSCIENCE AND BIOENGINEERING, (AUG 1999) Vol. 88, No. 2, pp. 130-135.
Publisher: SOC BIOSCIENCE BIOENGINEERING JAPAN, OSAKA UNIV, FACULTY ENGINEERING, 2-1 YAMADAOKA, SUITA, OSAKA 565-0871, JAPAN.
ISSN: 1389-1723.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 22

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A beta-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding beta-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other beta-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to beta-mannosidase from *P. furiosus* (55.7%), beta-**glycosidase** from *Sulfolobus solfataricus* (42.7%) and beta-glucosidase from *P. furiosus* (41.9%). The cloned gene was expressed in *Escherichia coli* and the recombinant protein was purified. The beta-**glycosidase** showed optimal activity at pH 6.5 and at an extremely high temperature of 100 degrees C, and had a half-life of 18 h at 90 degrees C. The beta-**glycosidase** hydrolyzed various pNp-beta-glycopyranosides, with k(cat)/K-m values in the order of pNp-beta-glucopyranoside is approximately equal to pNp-beta-mannopyranoside > pNp-beta-galactopyranoside > pNp-beta-xylopyranoside. pNp-beta-mannopyranoside was the substrate exhibiting the lowest K-m value [0.254 mM] with a k(cat)/K-m ratio comparable to that of pNp-beta-glucopyranoside. This substrate specificity was distinct from

previously reported **beta-glycosidases**. We observed that the region in Pk-Gly corresponding to the first alpha-helix and beta-strand region of **beta-glycosidase** from *S. solfataricus*, which constitutes a large portion of the channel for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to **beta-glycosidases** and the C-terminal region similar to **beta-mannosidases**. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L20 ANSWER 12 OF 15 SCISEARCH COPYRIGHT 2000 ISI (R)

ACCESSION NUMBER: 96:698965 SCISEARCH

THE GENUINE ARTICLE: VJ442

TITLE: COMPARISON OF A BETA-GLUCOSIDASE AND A BETA-MANNOSIDASE FROM THE HYPERTHERMOPHILIC ARCHAEON **PYROCOCCLUS** -FURIOSUS - PURIFICATION, CHARACTERIZATION, GENE CLONING, AND SEQUENCE-ANALYSIS

AUTHOR: BAUER M W; BYLINA E J; SWANSON R V; KELLY R M (Reprint)

CORPORATE SOURCE: N CAROLINA STATE UNIV, DEPT CHEM ENGN, RALEIGH, NC, 27695 (Reprint); N CAROLINA STATE UNIV, DEPT CHEM ENGN,

RALEIGH, NC, 27695; RECOMBINANT BIOCATALYSIS INC, SHARON HILL, PA, 19079

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 SEP 1996) Vol. 271, No. 39, pp. 23749-23755. ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 78

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus** furiosus: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) *Biochem. J.* 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) *J. Bacteriol.* 177, 7105-7111; GenBank accession no. U37557).

The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the

beta-**glycosidases** from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities,

the beta-mannosidase and the beta-glucosidase can both be classified as family

1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from *P. furiosus* both contained the conserved active

site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at pH 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at 100 degrees C. K-m and V-max values for the beta-mannosidase were determined to be 0.79 mM and 31.1 mu mol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the P.

Pyrococcus furiosus beta-glucosidase (5.3 versus 4, 500 s(-1) m(-1) with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:95843 HCAPLUS
TITLE: Novel substrate specificity of a membrane-bound .beta.-glycosidase from the hyperthermophilic archaeon *Pyrococcus horikoshii*
AUTHOR(S): Matsui, I.; Sakai, Y.; Matsui, E.; Kikuchi, H.; Kawarabayashi, Y.; Honda, K.
CORPORATE SOURCE: National Institute of Bioscience and Human-Technology,
Tsukuba, Ibaraki, Japan
SOURCE: FEBS Lett. (2000), 467(2,3), 195-200
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A .beta.-glycosidase gene homolog of *Pyrococcus horikoshii* (BGPh) was successfully expressed in *Escherichia coli*. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85.degree.C for 15 min. The optimum pH was 6.0 and the optimum temp. was over 100.degree.C, resp. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90.degree.C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with kcat/Km values high enough for hydrolysis of .beta.-D-Glcp derivs. with long alkyl chain at the reducing end and low enough for the hydrolysis of .beta.-linked glucose dimer more hydrophilic than aryl- or alkyl-.beta.-D-Glcp.

L20 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:590680 HCAPLUS
DOCUMENT NUMBER: 125:268847
TITLE: Comparison of a .beta.-glucosidase and a .beta.-mannosidase from the hyperthermophilic archaeon *Pyrococcus furiosus*. Purification, characterization, gene cloning, and sequence analysis
AUTHOR(S): Bauer, Michael W.; Bylina, Edward J.; Swanson, Ronald V.; Kelly, Robert M.
CORPORATE SOURCE: Dep. Chem. Eng., North Carolina State Univ., Raleigh, NC, 27695-7905, USA
SOURCE: J. Biol. Chem. (1996), 271(39), 23749-23755
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two distinct exo-acting, .beta.-specific glycosyl hydrolases were purified to homogeneity from crude cell exts. of the hyperthermophilic archaeon *Pyrococcus furiosus*: a .beta.-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a .beta.-mannosidase. The .beta.-mannosidase and .beta.-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calcd. mol. masses of 59.0 and 54.6 kDa for the .beta.-mannosidase and the .beta.-glucosidase, resp. The .beta.-glucosidase gene was identical to that reported by Voorhorst

et

al. (Voorhorst, W. L. B., Eggen, R. I. L., Luesink, M. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557). The deduced amino acid sequences showed homol. both with each other (46.5% identical) and with several other glycosyl hydrolases, including the .beta.-**glycosidases** from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities, the .beta.-mannosidase and the .beta.-glucosidase can both be classified as family 1 glycosyl hydrolases. In addn., the .beta.-mannosidase and .beta.-glucosidase from *P. furiosus* both contained the conserved active site residues found in all family 1 enzymes. The .beta.-mannosidase showed optimal activity at pH 7.4 and 105.degree.C. Although the enzyme had a half-life of greater than 60 h at

90.degree.C, it is much less thermostable than the .beta.-glucosidase, which had a reported half-life of 85 h at 100.degree.C. Km and Vmax values for the .beta.-mannosidase were detd. to be 0.79 mM and 31.1 .mu.mol para-nitrophenol released/min/mg with p-nitrophenyl-.beta.-D-mannopyranoside as substrate. The catalytic efficiency of the .beta.-mannosidase was significantly lower than that reported for the *P. furiosus* .beta.-glucosidase (5.3 vs. 4, 500 s⁻¹ mM⁻¹ with p-nitrophenyl-.beta.-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the .beta.-glucosidase, the primary role of the .beta.-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L20 ANSWER 15 OF 15 LIFESCI COPYRIGHT 2000 CSA

ACCESSION NUMBER: 97:345 LIFESCI

TITLE: Comparison of a beta -glucosidase and a beta -mannosidase

from the hyperthermophilic archaeon **Pyrococcus furiosus**: Purification, characterization, gene cloning,

and

sequence analysis

AUTHOR: Bauer, M.W.; Bylina, E.J.; Swanson, R.V.; Kelly, R.M.*

CORPORATE SOURCE: Dep. Chem. Eng., North Carolina State Univ., Raleigh, NC 27695-7905, USA

SOURCE: J. BIOL. CHEM., (1996) vol. 271, no. 39, pp. 23749-23755. ISSN: 0021-9258.

DOCUMENT TYPE: Journal

FILE SEGMENT: N; G; J

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Two distinct exo-acting, beta -specific glycosyl hydrolases were purified

to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus furiosus**: a beta -glucosidase, corresponding to the one previously purified by Kengen et al., and a beta -mannosidase. The beta -mannosidase and beta -glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta -mannosidase and the beta -glucosidase, respectively. The beta -glucosidase gene was identical to that reported by Voorhorst et al. The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the

beta

-**glycosidases** from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities,

the

beta -mannosidase and the beta -glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta -mannosidase and beta -glucosidase from *P. furiosus* both contained the conserved active site residues found in all family 1 enzymes. The beta -mannosidase

showed

JOURNAL code: EOH. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000602

AB A **beta-glycosidase** gene homolog of **Pyrococcus** horikoshii (BGPh) was successfully expressed in *Escherichia coli*. The enzyme was localized in a membrane fraction and solubilized with 2.5% Triton X-100 at 85 degrees C for 15 min. The optimum pH was 6.0 and the optimum temperature was over 100 degrees C, respectively. BGPh stability was dependent on the presence of Triton X-100, the enzyme's half-life at 90 degrees C (pH 6.0) was 15 h. BGPh has a novel substrate specificity with k(cat)/K(m) values high enough for hydrolysis of beta-D-Glcp derivatives with long alkyl chain at the reducing end and low enough for the hydrolysis of beta-linked glucose dimer more hydrophilic than aryl- or alkyl-beta-D-Glcp.

L21 ANSWER 2 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 1999327626 EMBASE
TITLE: Gene analysis and enzymatic properties of thermostable .beta.-**glycosidase** from **Pyrococcus** kodakaraensis KOD1.
AUTHOR: Ezaki S.; Miyaoku K.; Nishi K.-I.; Tanaka T.; Fujiwara S.; Takagi M.; Atomi H.; Imanaka T.
CORPORATE SOURCE: T. Imanaka, Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
SOURCE: Journal of Bioscience and Bioengineering, (1999) 88/2 (130-135).
Refs: 22
ISSN: 1389-1723 CODEN: JBBIF6
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A .beta.-**glycosidase** with broad substrate specificity was identified from a hyperthermophilic archaeon, **Pyrococcus** kodakaraensis KOD1. The gene encoding .beta.-**glycosidase** (Pk-gly) consists of 1449 nucleotides corresponding to a polypeptide of 483 amino acids. The protein showed similarity with other .beta.-**glycosidases** from family-1 glycosyl hydrolases, in particular, it showed high identity to .beta.-mannosidase from *P. furiosus* (55.7%), .beta.-**glycosidase** from *Sulfolobus solfataricus* (42.7%) and .beta.-glucosidase from *P. furiosus* (41.9%). The cloned gene was expressed in *Escherichia coli* and the recombinant protein was purified. The .beta.-**glycosidase** showed optimal activity at pH 6,5 and at an extremely high temperature of 100.degree.C, and had a half-life of 18 h at 90.degree.C. The .beta.-**glycosidase** hydrolyzed various pNp-.beta.-glycopyranosides, with k(cat)/K(m) values in the order of pNp-.beta.-glucopyranoside dot left right equal to pNp-.beta.-mannopyranoside>pNp-.beta.-galactopyranoside>pNp-.beta.-xylopyranoside. pNp-.beta.-mannopyranoside was the substrate exhibiting the lowest K(m) value [0.254 mM] with a k(cat)/K(m) ratio comparable to that of pNp-.beta.-glucopyranoside. This substrate specificity was distinct from previously reported .beta.-**glycosidases**. We observed that the region in Pk-Gly corresponding to the fifth .alpha.-helix and .beta.-strand region of .beta.-**glycosidase**

from *S. solitarius*, which constitutes a large portion of the channel

• for substrate incorporation, displayed a chimeric structure, with the N-terminal region similar to .beta.-glycosidases and the C-terminal region similar to .beta.-mannosidases. An exo-type hydrolytic activity and transglycosylation activity were also observed towards cellooligomers.

L21 ANSWER 3 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97196266 EMBASE
DOCUMENT NUMBER: 1997196266
TITLE: Cloning, sequencing, characterization, and expression of an
extracellular .alpha.-amylase from the hyperthermophilic archaeon *Pyrococcus* furiosus in *Escherichia coli* and *Bacillus subtilis*.
AUTHOR: Jorgensen S.; Vorgias C.E.; Antranikian G.
CORPORATE SOURCE: G. Antranikian, Inst. of Biotechnology, Dept. of Technical Microbiology, Technical University Hamburg-Harburg, Denickestr. 15, 21071 Hamburg, Germany
SOURCE: Journal of Biological Chemistry, (1997) 272/26 (16335-16342).
Refs: 27
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A gene encoding a highly thermostable extracellular .alpha.-amylase from the hyperthermophilic archaeon *Pyrococcus* furiosus was identified. The gene was cloned, sequenced, and expressed in *Escherichia coli* and *Bacillus subtilis*. The gene is 1383 base pairs long and encodes

a protein of 461 amino acids. The open reading frame of the gene was verified by microsequencing of the recombinant purified enzyme. The deduced amino acid sequence is 25 amino acids longer at the N terminus than that determined by sequencing of the purified protein, suggesting that a leader sequence is removed during transport of the enzyme across the membrane. The recombinant .alpha.-amylase was biochemically characterized and shows an activity optimum at pH 4.5, whereas the optimum temperature for enzymatic activity is close to 100 .degree.C. .alpha.-Amylase shows sequence homology to the other known .alpha.-amylases and belongs to family 13 of glycosyl hydrolases. This extracellular .alpha.-amylase is not homologous to the subcellular .alpha.-amylase previously isolated from the same organism.

L21 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 96394494 MEDLINE
DOCUMENT NUMBER: 96394494
TITLE: Comparison of a beta-glucosidase and a beta-mannosidase from the hyperthermophilic archaeon *Pyrococcus* furiosus. Purification, characterization, gene cloning, and
sequence analysis.
AUTHOR: Bauer M W; Bylina E J; Swanson R V; Kelly R M
CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Sep 27) 271 (39) 23749-55.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals

AB Two distinct exo-acting, beta-specific glycosyl hydrolases were purified to homogeneity from crude cell extracts of the hyperthermophilic archaeon **Pyrococcus furiosus**: a beta-glucosidase, corresponding to the one previously purified by Kengen et al. (Kengen, S. W. M., Luesink, E. J., Stams, A. J. M., and Zehnder, A. J. B. (1993) Eur. J. Biochem. 213, 305-312), and a beta-mannosidase. The beta-mannosidase and beta-glucosidase genes were isolated from a genomic library by expression screening. The nucleotide sequences predicted polypeptides with 510 and 472 amino acids corresponding to calculated molecular masses of 59.0 and 54.6 kDa for the beta-mannosidase and the beta-glucosidase, respectively. The beta-glucosidase gene was identical to that reported by Voorhorst et al. (Voorhorst, W. G. B., Eggen, R. I. L., Luesink, E. J., and deVos, W. M. (1995) J. Bacteriol. 177, 7105-7111; GenBank accession no. U37557U37557). The deduced amino acid sequences showed homology both with each other (46.5% identical) and with several other glycosyl hydrolases, including the beta-glycosidases from *Sulfolobus solfataricus*, *Thermotoga maritima*, and *Caldocellum saccharolyticum*. Based on these sequence similarities, the beta-mannosidase and the beta-glucosidase can both be classified as family 1 glycosyl hydrolases. In addition, the beta-mannosidase and beta-glucosidase from *P. furiosus* both contained the conserved active site residues found in all family 1 enzymes. The beta-mannosidase showed optimal activity at pH 7.4 and 105 degrees C. Although the enzyme had a half-life of greater than 60 h at 90 degrees C, it is much less thermostable than the beta-glucosidase, which had a reported half-life of 85 h at 100 degrees C. Km and Vmax values for the beta-mannosidase were determined to be 0.79 mM

and

31.1 micromol para-nitrophenol released/min/mg with p-nitrophenyl-beta-D-mannopyranoside as substrate. The catalytic efficiency of the beta-mannosidase was significantly lower than that reported for the *P. furiosus* beta-glucosidase (5.3 versus 4, 500 s⁻¹ mM⁻¹ with p-nitrophenyl-beta-D-glucopyranoside as substrate). The kinetic differences between the two enzymes suggest that, unlike the beta-glucosidase, the primary role of the beta-mannosidase may not be disaccharide hydrolysis. Other possible roles for this enzyme are discussed.

L21 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1997:170983 BIOSIS
DOCUMENT NUMBER: PREV199799477586
TITLE: A new thermostable glucose-activated beta-glucosidase from the hyperthermophilic marine archaeobacterium **Pyrococcus abyssi**: Purification and characterization.
AUTHOR(S): Ladrat, Christine (1); Alayse-Danet, Anne-Marie; Dietrich, Jacques; Barbier, George
CORPORATE SOURCE: (1) Dep. Environ. Profond, IFREMER, BP 70, 29280 Plouzane France
SOURCE: Journal of Marine Biotechnology, (1996) Vol. 4, No. 4, pp. 192-199.
ISSN: 0941-2905.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A thermostable beta-glucosidase from the deep-sea hyperthermophilic archaeobacterium **Pyrococcus abyssi** strain ST549 has been purified to apparent homogeneity and characterized. Production of beta-glucosidase by this strain was stimulated by the addition of cellobiose to the culture medium. This enzyme was homodimeric, each subunit having a molecular weight of 64 kDa. Its isoelectric point was 4.3. The enzyme showed maximal activity at pH 6 and 100 degree C and was highly thermostable. It exhibited different beta-glycosidase

activities and hydrolyzed short-chain oligosaccharides. Chemical modifications of amino acids indicated that no accessible sulfhydryl group was essential for activity and that histidine and tryptophan were probably involved in the maintenance of the integrity of the active site. In marked contrast to others presently known, the beta-glucosidase from the ST549 strain was activated by glucose and polyols. This property suggests specific interactions between the enzyme and polyhydric alcohols and the probable occurrence of a transglycosylation mechanism. This mechanism and potential applications are discussed.

L21 ANSWER 6 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 90219204 EMBASE

DOCUMENT NUMBER: 1990219204

TITLE: Purification and characterization of an .alpha.-glucosidase

from a hyperthermophilic archaebacterium, **Pyrococcus furiosus**, exhibiting a temperature optimum of 105 to 115.degree.C.
AUTHOR: Costantino H.R.; Brown S.H.; Kelly R.M.
CORPORATE SOURCE: Department of Chemical, Engineering, Johns Hopkins University, Baltimore, MD 21218, United States
SOURCE: Journal of Bacteriology, (1990) 172/7 (3654-3660).
ISSN: 0021-9193 CODEN: JOBAAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Pyrococcus furiosus** is a strictly anaerobic hyperthermophilic archaebacterium with an optimal growth temperature of about 100 .degree.C. When this organism was grown in the presence of certain complex carbohydrates, the production of several amylolytic enzymes was noted. These enzymes included an .alpha.-**glycosidase** that was located in the cell cytoplasm. This .alpha.-glucosidase has been purified 310-fold and corresponded to a protein band of 125 kilodaltons as

resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The enzyme exhibited optimum activity at pH 5.0 to 6.0 and over a temperature range of 105 to 115.degree.C. Kinetic analysis conducted at 108.degree.C revealed hydrolysis of the substrate p-nitrophenyl-.alpha.-D-glucopyranoside (PNPG), methyl-.alpha.-D-glucopyranoside, maltose, and isomaltose. Trace activity was detected towards p-nitrophenyl-.beta.-D-glucopyranoside, and no activity could be detected towards starch or sucrose. Inhibition studies conducted at 108.degree.C with PNPG as the substrate and maltose as the inhibitor yielded a K(i) for maltose of 14.3 mM. Preincubation for 30 min at 98.degree.C in 100 mM dithiothreitol and 1.0 M urea had little effect on enzyme activity, whereas preincubation in 1.0% sodium dodecyl sulfate and 1.0 M guanidine hydrochloride resulted in significant loss of enzyme activity. Purified .alpha.-**glycosidase** from *P. furiosus* exhibited remarkable thermostability; incubation of the enzyme at 98.degree.C resulted in a half life of nearly 48 h.